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REVIEW ARTICLE

DEVELOPMENT OF PRODUCT CONTAINING MICROENCAPSULATED PROBIOTICS: AN UPDATE ON ISSUES

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ABSTRACT

To have a handy reference and a source of outstanding knowledge for the scientist engaged in developing products containing probiotics (PRs). Probiotic micro-organisms explored for delivering associated proclaimed valuable benefits. PRs included in the pharmaceutical, dairy, nondairy, and personal care products. The market of all these products is expanding day by day, which expected to prosper. Diverse methods and technology devised to get their product with wished and improved performances. All these methods or technologies have failed in achieving preset goal, as they could not improve their performances and marketplace survival. Microencapsulation (MEC) can achieve goals with promoted degrees of success. Interest evoke for marketing of product containing encapsulated probiotics (PCMP), as means to upkeep their performance, reproducible, throughout life cycle. Related reports are available in literatures, which are unable to provide latest information and found unhandy for professionals. In this regard, information collected from form databases and presented as a handy reference. The review features on basis, limits, and applicability of MEC method suited for evolution of PCMP. Theme of debate also covers issues on their development, evaluation, and marketing. Presented information will be a helping hand for scientists and will offer an outstanding knowledge to developers, while designing them, with excellent feature and improved marketability.

Key words: Development, probiotics, processing, product, quality, safety.

INTRODUCTION

Intake of PRs, in enough quantity and for stated period, improves health, well-being, and wealth of the consumer [1, 2]. Nowadays, their products pushed up for curing illness, and improving health and eudemonia [3, 4]. Raised health care cost and alert for personal health, evolution of antibiotics resistance, hope for longer life, lifestyle changes, and self-medication revolutionized their use [1, 5]. Superior knowledge and sophistication in technology, and strife overturned their market [2]. Research in the area has heading for making them basis for healthy civilization, overcoming disease, improving safety linked to them, and so on [1]. Research is underway to develop and assess their strain, and devising and finding industrial application of those strains with stood-out benefits. Besides, research afoot to get various products containing probiotics (PCP) with diverse role in marketplace, which can offer new choice to the demand and need of all types of consumers [6, 7]. Their market is expanding systematically, in diverse domains. Further, continuing innovation and rivalry have potency to create novel PCP [3, 4, 8].

The term "probiotics" coined in 1965 by Lilly and Stillwell while its definition has changed through the years. It defined, in present times, as "live micro-organisms which when administered in adequate amounts confer a health benefit on the host" [9]. Current technological progression, in these fields, devised to [7, 8, 10, 11]:

1. Assess their efficacy, single-strain or combination of multiple-strains,
2. Evaluate effect of prebiotics on their efficacy, single-strain- or combination of multiple-strains, and or
3. Present PCP in various domain and or form.

Science in these fields is budding in various domains. Turning this science into consumable create difficulties for regulatory bodies and scientific community. Side-by-side also imposing challenges on manufacturers [11, 12]. Besides, product design consists of multistage and expensive steps and too time-consuming [1].

Developing product is of four stages. Stage-I: evolution of scheme, Stage-II: design and evolution of method, Stage-III: launch and evaluation, and Stage-IV: commercialization. Figure 1 presents the stages of food products evolution.

Many reports published facts about boom in the variety of PCP and discrepancies about labeled claims and misidentification [12, 13]. These discrepancies considered to be arising because of execution of measly quality control. Which, thus, have deprived consumer's reliability on the marketed PCP [13, 14]. Set up and follow of method, for evaluating quality and worthwhile claims, based on measly scientific consensus, expanded awareness of researchers for regulating their marketing [12-14]. These state of affairs led to compliance of stricter regulations, territorial and global. Territorial and global controls, working in fragments, influence their marketing also creating disarray [11, 12, 15].

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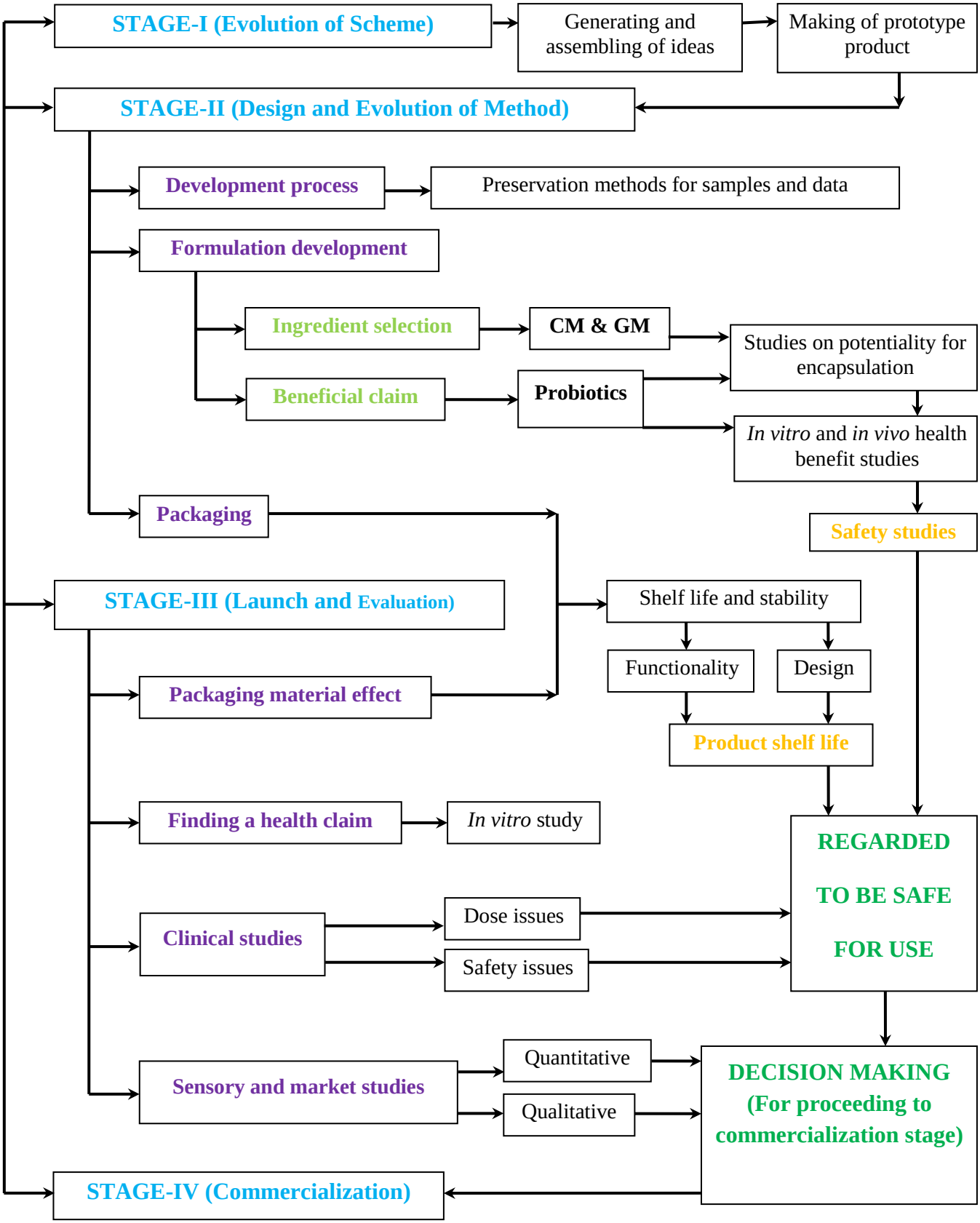

Figure 1: Stages of food products evolution

Where: CM stands for carrier matrix forming materials, and GM for gastro resistant enteric coating materials

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Needs of territorial and global rule states that while proclaiming their health claim and probiotic use, manufacturer has to consider [14, 15]:

(i) Viability of probiotic cells (VoPC) within product, and
(ii) Safety issues of product, even at the end of shelf life.

As a result, manufacturers devising approaches to prolong VoPC within PCP and develop smart packages [7, 16]. Smart packages developed to preserve their quality and safety and signal discrepancies [17].

For eliciting worthwhile benefit, viable-probiotics cells (VPC) must available in enough amounts in the intestine (at least, 10^9 CFU). There, they should multiply to achieve satisfactory gut colonization, above stated threshold level [12, 18].

Environment of making up and noncompliance to storage needs, during freight and storage, drop-off VoPC within PCP [7, 10]. After oral administration of PCP, hydrolytic-enzymes, acidic environment of stomach and bile salts in gastrointestinal (GI) tract decline VoPC [10].

Diverse approaches undertaken to protect VoPC during processing and storage, and their GI transit. Preferentially, adopted approach includes [7, 16, 19, 20]:

(i) Manipulation of fermentation and storage conditions of food carriers,
(ii) Careful selection of culture organisms,
(iii) Addition of different growth promoters, protectant and prebiotics in formula,
(iv) MEC, so on.

Above approaches have divergent degree of successes. Some approaches unable to fit in high numbers of VPC within PCP. While other fails in conserving VoPC within PCP, throughout its shelf life and GI-transit phase [7, 10].

Current scientific proof reveals that, VoPC within intestine could be sustaining by their MEC and improving their growth rate to lifted level referring peristalsis rate [7].

Nowadays, MEC receiving interests to conserve VoPC within shelf life and during GI-transit phase of PCP, from scientific community [7].

MEC of VPC potentiates *in vivo* action of PCP, whereas adding prebiotics, protectant, or both in formula further extend it. Later apply of gastro-resistant enteric coating (GREC), on microcapsules (MCs), give gastro-protection to encapsulated VPC side by side potentiate *in vivo* effect [21-25]. MEC synergizes efficiency and effectiveness of PCMP [7, 10]. Stated synergistic effects postulated to be attributable to follow facts associated with MEC:

1. MEC protects VoPC from damaging environments (causes such as, hydrolytic-enzymes, bile salts, acidic environments of stomach, storage condition, and so on),
2. It sustains and modifies cell release, and improves patient compliance, and
3. Besides, it extends shelf life and improves stability of PCP.

On the contrary, MEC affects organoleptic properties of product. In majority cases, process of encapsulation (PEN) deprives aesthetic features of PCMP while improve same, rarely. Delivered aesthetic features depend on aesthetic characters of encapsulating material [8, 26].

The PEN is complicate, challenging, and costly affair. The quality and quantity of VPC, and wished quality and stability of PCMP makes the method complicate and challenging. However, all these facts decide efficacy and efficiency of MEC method.

In setting of above, this review focuses updated information on background, issues, and technology and method adopted for evolution of PCMP. Method for their evaluation and improving their performances and marketing potential also discussed. The author believes informative content of this paper will be useful for the scientific community of academic and industrial field. Besides, an outstanding knowledge and application of proper MEC technologies ought to be enough to help developer to get PCMP, with excellent performance.

MICROENCAPSULATION OF PROBIOTICS

Encapsulation, a physicochemical or mechanical process, enables entrapment of a substance in a material, in order, to make particles have diameter ranging from nanometers to millimeters. Encapsulation yielding particles with few micrometer sizes called as "MEC" [7]. PEN involves sequential multiple steps. This starts from scattering of core material in a carrier matrix forming material (CMFM) and ends with cascading the core material with CMFM.

PEN involves sequential steps as follows [7]:

1. Preparing a solution, dispersion, or agglomerate, by adding core material to CMFM(s) solution;
2. Stabilizing resulting system;
3. Developing an encapsulate, through chemical (polymerization) or physical (evaporation, solidification, coalescence) or physicochemical (gelification) process; and
4. Hardening of encapsulate.

Studies reveal that encapsulation, polymeric, shield VPC from harmful environments of the GI tract, and improve their intestinal persistency and multiplication. Further, PCMP posses improved patient compliance, extended shelf life, and sustained and modified release profile, thus may be an innovative or have a new role [27]. Later application of GREC, as monolayer or layer-by-layer of nanolayers on prepared MCs of PCs conserves VoPC, on oral administration. This also improves its amenability for high water contented PCMP and shelf life [28, 29].

The quality and quantity of PRs strain and their upkeep of viability influence choice of PEN. Further, PEN decides characteristics of MCs by particle shape, size, and size range. Coating method, PEN, and GREC influence efficiency of VoPC upholding during processing, storage, and GI-transit phase of PCMP [7]. Co-encapsulating VPC with prebiotics and adding protectant in encapsulating wall material improve stability and performances of PCMP [7, 11, 16, 19].

OBJECTIVE AND GOAL

The preset objective and goal of PEN linked with PCMP are under [7].

1. To protect VoPC in harsh environment of stomach and destructive storage conditions;
2. Sustaining and releasing VPC, in plenty amounts (more than, 10^9 CFU) and in metabolically active state, during the intestinal phase, for achieving enough gut colonization and conferring health benefit to the host;
3. To pass on binding, complexing, or adherence ability to released VPC with target site, molecule, or micro-organism or to absorb target molecule or micro-organism in intestine;
4. Getting MCs, water-insoluble, that will preserve its integrity within PCMP;
5. Getting MCs with diverse properties having ability to got incorporate in a wide range of products such as tablets, capsules, ice-cream, yogurts, chocolates, and so on; and
6. To have MCs holding satisfactory stability within PCMP, this could be improving shelf life.

GREC MATERIALS AND CMFMS

Wide range of materials used, as GREC materials and CMFMs, but the use of biodegradable polymer will be worthwhile [30]. CMFM used are alginate, alginate-chitosan, gellan gum, xanthan gum, vegetable gums, carrageenan, sugars, starch, modified starch, dextrins, cellulose derivatives, hypromellose, caseinates, gelatin, glyceride derivatives, waxes, fats, milk proteins, zein and other proteins, and so on. While, commonly used GREC material are palm oil, poly-L-lysine, carboxymethyl cellulose, chitosan, hypromellose phthalate, cellulose acetate phthalate, and so on [30, 31]. Among them, food proteins and polysaccharides considered being the biodegradable polymer.

Exopolysaccharides; such as xanthan- or gellan- or pullulan-gum, and jamilan; could extend or modify release profile. Blend of xanthan or jamilan gum and gellan gum, alginate and xanthan or gellan gum can mend gastro-protective feature. Further, these could be achieving colon targeting [24, 25, 30]. Calcium alginate preferred as CMFM as is biocompatible, non-toxic, and cheaper. Besides, it involves simple processing steps [30]. Hydrogel of alginate yields porous MCs of PRs but unable to conserve VoPC during GI transit phase. This needs 60-80 °C to prepare watery solution of alginate and creates scale-up problems. These problems can overcome by cascading prepared MCs with GREC material [7]. While, blending alginate with starch or chitosan and its structural modification can overcome same [32]. Starch and resistant starches can deliver, efficiently, VPC in the large intestine. Their prebiotics role ought to holds synergistic action. Surface feature of resistant starch could result adhesion of probiotics cells (PCs), to its surface. This features of resistant ensuring betterment of VoPC and intestinal targeting [33]. Gelatin alone or in blend with gellan gum or other material, although, used as CMFM but unable to offer gastro-protection [30]. Whey proteins and milk proteins, biocompatible polymers, improve aesthetic feature and hold excellent gelation character [34-36]. Hypromellose can control release profile of PCs, extend their gastric retention time, and improve their gut colonization [18]. Gelification feature of carrageenan could result entrapment of PCs. Needs for blending of dispersion of PCs with heat-sterilized suspension at 40-45 °C, is damaging. Which in-turn increase in-process loss of viability of probiotics (iPVLP). Besides, it owes approval, from several government agencies, as a food additive [30]. However, chitosan haves inhibitory effects on LAB but can deliver VPC to colon [28]. Further, cellulose acetate phthalate and hypromellose phthalate reckoned as safe and yield MCs, water insoluble one [7, 18, 31].

General recognition is that biodegradable-biopolymers can deliver VPC at site of action. They can alter look, texture, stability, and flavor of PCMP but slightly affect their physicochemical features. Basing on wanted features of PCMP, biopolymer particles ought to crafted with wished features. Mended feature linked to rheology, optic, encapsulation, release profile, and physicochemical stability. Designing of biopolymer did by exploiting correct monomers, biopolymers, particle-creation method, polymerization method, and finishing steps. Commonly used food proteins and polysaccharides exploited for making up particulate-system. Particles developed following extrusion, gelation or solvent removal technique. Biopolymer based particulate conception method considered unsuited, either because of materials of nonfood-grade or increased costs of processing [37].

METHOD AND PROCEDURE

Technological choices existing for MEC are physical methods such as air-suspension coating, pan coating, spray-drying, vibrational nozzle, centrifugal extrusion. Substitute methods are chemical method such as in situ-, interfacial-, or matrix-polymerization, simple and complex coacervation, and so on. Among available methods, MEC of PCs done with spray drying, spray freeze-drying and impinging aerosol. Other methods are extrusion, fluidized-bed coating and agglomeration, and direct compression encapsulation. Reserve methods are dispersion and ionic or enzymatic gelification, dispersion and interfacial-polymerization, inter-polymer complex encapsulation with supercritical carbon dioxide. Although these have applicability in getting PCMP but have, several limits like involvement of organic solvents and high-pressure and or temperature, scale-up problems, and so on. It must accent that none of these methods results shelf-stable MCs loaded with large numbers of VPC having lasting viability throughout shelf life [7, 38]. Succeeding sections provide method, leaning and limits of methods to get PCMP. Figure 2 shows Method of evolution of encapsulation process employed for PRs.

SPRAY DRYING METHOD (SDM). In SDM, dispersion of VPC, in watery solution of CMFM, subjected to spraying through a sprayer or atomizer and concurrent drying of formed droplets, by application of hot air, in specially designed closed-container, to get MCs. SDM create MCs with quickness, duplicability, and with low production cost. Superior scale-up potentiality and nonstop processing make it amenable for industrial scale. It fails in protecting iPVLP, which linked to simultaneous dehydration and thermal inactivation [10, 39]. While, iPVLP can be improve by adding protectants, such as, whey-protein, and resistant and N-Tack starch, in formula [33, 40].

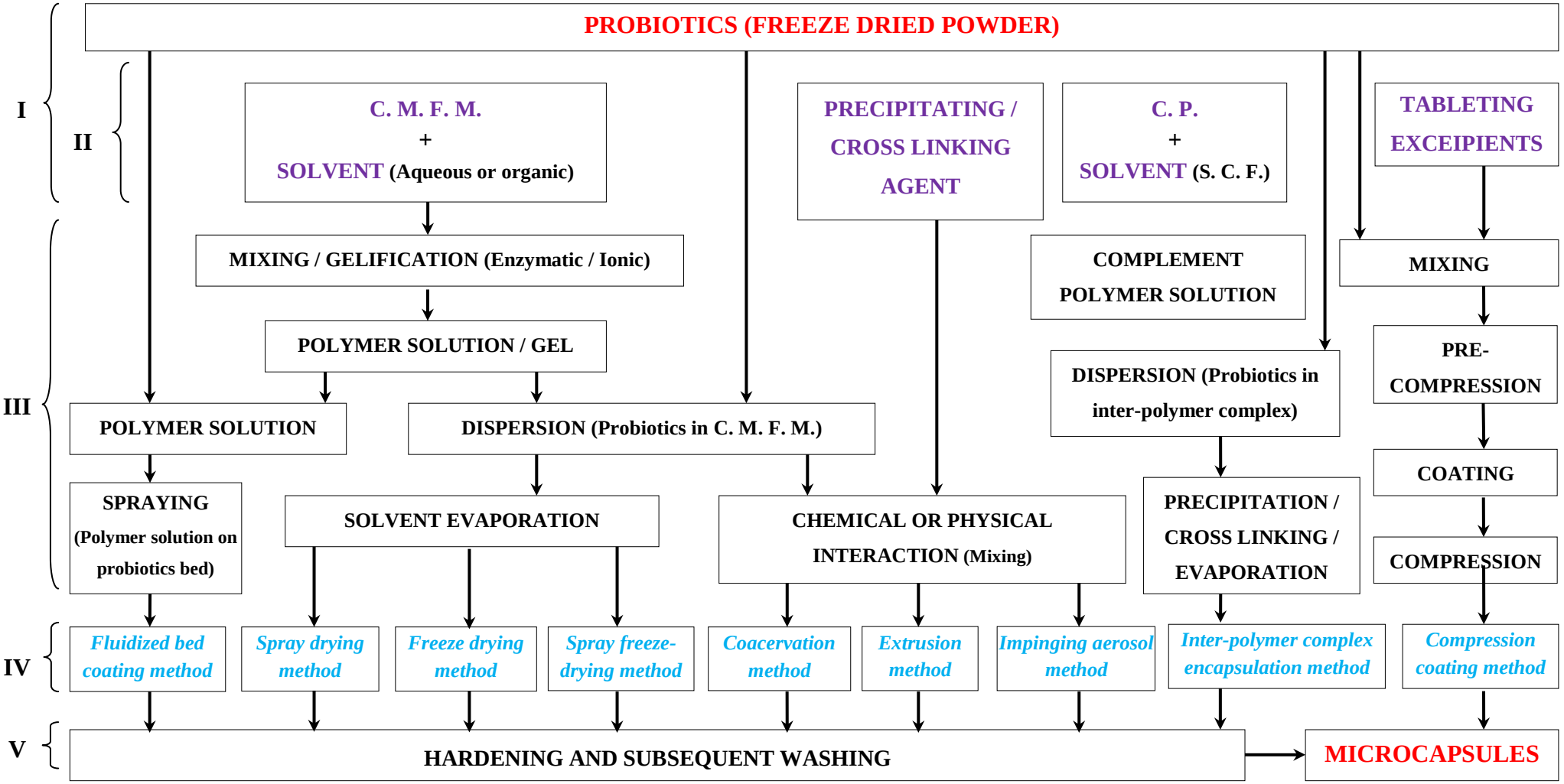

Figure 2-Method of evolution of encapsulation process employed for probiotics.

***Where**: Section I depicts ingredients, while that of II excipients, III intermediate steps, IV encapsulation methodology, and V final product. C. M. F. M. stands for carrier matrix forming material, C. P. for complement polymers and S. C. F. for super critical carbon dioxide fluid.*

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SPRAY FREEZE-DRYING METHOD (SFDM). SFDM involves processing steps parallel to freeze-drying and spray-drying while removes limit of spray drying. In SFDM, suspension of freeze-dried VPC (FVPC) atomized into a cold vapor phase of a cryogenic liquid (such as liquid nitrogen), to achieve a dispersion of frozen droplets which afterwards freeze-dried. SFDM result in MCs with controlled size and larger specific surface areas. Referring SDM, this employs high-energy and long processing time, have high iPVLP, and increases production cost by 30–50 times. Ability to control release profile and improved storage survivability at 4 °C heightens benefit [39, 41, 42]. High iPVLP issue of it improved by adding protectant, in formula, such as whey-protein, resistant starch, skimmed milk, and so on [33, 43].

IMPINGING AEROSOL METHOD (IAM). IAM involves impinging interactions of aerosols of alginate and aerosols of calcium chloride, from opposite directions within a chamber. As alginate solutions, atomized, droplet gels-out as they meet with calcium chloride aerosols and thus fall as MCs on bottom of the chamber. IAM involves scattering of FVPC, in alginate solution before atomization or aerosol formation [44, 45]. It holds continuous processing capacity and high scale-up potentiality, lessens iPVLP, and protects VoPC in high acid and bile environment, but costly.

DISPERSION AND IONIC-GELIFICATION METHOD (DIGM). DIGM employs alginate, carrageenan, or pectin as CMFM. These form hydrocolloid on interacting with ions [43]. After making hydrocolloid, FVPC scattered in it. After stabilizing dispersion, by adding surfactant, cross-linking or hardening agent added, into it, to get MCs. DIGM has high scale-up potential with low iPVLP, but yields smaller sized particles with diverse shape having a wider range of size distribution. High cost and scale-up problems make it unsuitable for commercial scale [10]. Layer-by-layer coatings of nanolayers self-assembly of polyelectrolytes, on MCs being did with this method [29].

The alginate-starch method involves interactions of alginate and starch, a CMFM, with Ca^{2+}, a gelling agent. Adding glycerol, in formula, augments survival of VPC during processing and storage. While inclusion of Hi-Maize starch, in formula, improve encapsulation efficiency [33]. In gelatin or gelatin-maltodextrin based system, gelatin or gelatin-maltodextrin employed as CMFM. While, genipin used as cross-linking agent, and alginate as GREC material. MCs prepared by interacting CMFM and cross-linking agent. Later application of GREC on them, with alginate, passes on gastro-protection to encapsulate. GREC did by cross-linking the alginate with Ca^{2+} following internal or external source method [46, 47]. Further, whey-proteins, in amphoteric state, on reaction with negatively charged polysaccharides can end with MEC of VPC. This scheme involves pH adjustment of dispersion, of whey-protein, to isoelectric point of whey proteins, for making hydrocolloid. Then polysaccharide (such as pectin, carrageenan, or alginate) added, which cause encapsulation followed by separation of MCs [48].

DISPERSION AND ENZYMATIC-GELIFICATION METHOD (DEGM). DEGM is an adapted version of DIGM. Contrary to DIGM, DEGM employs enzymes as gelling agent in place of ions. Further, this method employs rennet, an enzyme, for gelling of milk proteins, thus called "rennet gelification method." Concentrated watery solutions of milk protein, on gelling, result a high-density gel network, a favorable micro-milieu for encapsulated PCs, have thus improved applicability. Although, DEGM has improved feasibility for controlling particle size and has potential for augmenting aesthetic or sensory property but is unsuitable for large-scale productions. However, a prospering procedure resulting MCs that are water insoluble, have high entrapment efficiency and insignificant iPVLP, and achieve gastro-protection [7, 34, 36].

EXTRUSION (PULSATION OR VIBRATIONAL JET NOZZLE) METHOD (EPVM). EPVM called cold gelation method and uses hydrocolloids. Hydrocolloid got by ionic or enzymatic gelation of CMFM. CMFM used are alginate, carrageenan, milk proteins, whey proteins, and so on. Dispersion of FVPC, in hydrocolloid of CMFM, passed through a nozzle at high-pressure, in droplet form, into solution of cross-linking or hardening agent to form MCs [48]. Droplet prepared by pulsating or vibrating the jet nozzle. Besides, atomizers (in case of prilling technique) or electrostatic field used to get droplets. Alternatively, a coaxial flow system followed. Resulted MCs then coated with whey-protein, for improving aesthetic features; or alginate, for giving GREC. These coatings applied following simple immersion method [10, 48].

Whey-protein based scheme uses whey-protein (denatured) as CMFM. This involves extruding blend of whey-protein isolate and FVPC followed by adjusting pH of dispersion below isoelectric point of whey-proteins. Prepared MCs, having loading efficiency up to 96%, afterward loaded in PCMP. Extrusion considered as a cheap and simple method, which involves gentle operation and can carried out under aerobic and anaerobic condition. The method is capable in minimizing iPVLP but need for later application of GREC. Needs for GREC and scale-up problem make it unalienable for commercial scale [48, 49].

DISPERSION AND INTERFACIAL-POLYMERIZATION OR COMPLEX COACERVATION METHOD (DIPCCM). Current scientific innovations have devised a single step, called complex coacervation. DIPCCM involves preparation of dispersion consisting of dispersed phase (watery suspension of FVPC) and continuous phase (solution of CMFM in organic solvent). On stabilizing dispersion, encapsulation resulted by adding a soluble biocompatible agent into continuous phase. This encapsulates PCs within a thin and strong membrane [7, 50]. Watery coacervation and phase separation method expel exposure of PCs to non-aqueous solvent and heat. However, is unsuitable for commercial scale, while used to get mucoadhesive MCs in small-scale [7, 18, 31].

INTER-POLYMER COMPLEX ENCAPSULATION WITH SUPERCRITICAL CARBON DIOXIDE METHOD (ICESCF). Moolman *et al.* have patented a novel method, using supercritical CO_2 fluid (SCF) as a medium for polymer processing, involving H-bond associate inter-polymer complexation, for PCs encapsulation [51]. ICESCF involves dissolving each of complementary polymers at once in same SCF, or one by one to form separate complementary polymer solutions followed by mixing separate solutions together to interact contained complementary polymers, resulting inter-polymer complex solution in SCF. At least two complementary polymers,

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having ability to interact in solution phase, is desirable, to form an inter-polymer complex. FVPC scattered in the complementary solutions before or after dissolving the associated polymer before interaction or mixing step. Otherwise, FVPC scattered in inter-polymer complex solution before or after dissolving the inter-polymer complex, before precipitating inter-polymer complex. Precipitation could be achievable by either changing the pressure and or temperature of SCF or adding a non-solvent constituent. Otherwise, dispersion of FVPC, in inter-polymer complex solution, is concentrate by spray drying the same after its atomization. Low molecular weight alcohol and or poloxamer and or ethylene oxide-propylene oxide tri-block copolymer used as solubilizer for improving solubility of the complementary polymers and or of the inter-polymer complex in SCF [51]. Adding glyceryl monostearate in formula improves viability protecting efficiency of MCs while polycaprolactone and ethylene oxide-propylene oxide tri-block copolymer decreases the same [38, 51]. The method encapsulates VPC with miserly iPVLP. However, hold low scale-up potentiality and costlier [38, 51].

FLUIDIZED-BED COATING AND AGGLOMERATION PROCESS (FCAP). FCAP, an advanced method, involve spraying solution of GREC material onto FVPC bed, kept under fluidized state in fluidized-bed processor (FBP) [7, 10]. An advancement of technological substantiation, done by Lallemand group of Canada, recorded as patent. That involves GREC of freeze-dried LAB with fatty acids [52]. This invention protects encapsulated PCs from harsh effects of temperature, gastric pH, and compression [52]. Cell Biotech has patented a dual coating technology for LAB (Patent number: EU 1514533). This involves coating of LAB with soy peptides followed by cellulose and gum. The method prevents the loss of VoPC during processing, storage, and GI transit. Further, FCAP hold high reproducibility and adapted to give multilayer coatings. It is suitable for giving GREC to prepared MCs within same equipment. The method owes high scale-up potential and is amenable for commercial scale. Associated high iPVLP, higher processing cost, and improved mastering difficulty considered as major disadvantages [7, 10].

DIRECT COMPRESSION ENCAPSULATION METHOD (DCEM). DCEM involves pre-compression of FVPC and excipient blend into pellets. Latter on the pellets are give GREC and finally compressed as tablets. GREC has done in FBP, with blend of sodium alginate and hydroxypropyl cellulose. While compression done at pressures up to 90 MPa. It considered cheapest method that designed for commercial scale production and improving shelf life. DCEM has lower entrapment efficiency and higher iPVLP. It slows down VPC release in intestinal conditions. While an increase in compression pressure, decrease VoPC above 90 MPa [7, 53].

IN-PROCESS EVALUATION

COMPLIANCE OF REGULATORY WANTS FOR USE OF PROBIOTICS AND ADDITIVES. Microbial strains with valid identity and genetic stability, filed in dossier filed to regulators, should used in evolution of formula or PCMP [15, 54-56]. FVPC subjected to species and strain determination test, viable cell count test, cell count tests (bacteriological, total aerobic bacteria, *Coliforms*, enterobacteria and other gram negative bacteria, and Yeast and Molds), and ensuring absence of contaminants [15, 31].

Indeed, laws governing food production and use of food additive has grown complex, which mandates their safety assessment [57, 58]. CMFMs, GREC materials, solvents, and additives used in formula or during evolution of PCMP should hold consent from regulators. Besides, they must complying specification for food contact substances limit, estimated daily intake limit, and acceptable daily intakes limit [57]. Coating of feed supplement loaded MCs with alginate, carrageenan, gellan or xanthan restrained in some countries thus have to exclude from formula [30].

EVALUATION OF MICROCAPSULES. Performance of MCs, loaded with VPC, comprises most important ingredient of PCMP, will oversee and control its performance. Studies reveal that physical properties of MCs, loaded with VPC, influences VoPC, during storage [40]. Thus, for evaluating and up keeping inter-batch and intra-batch reproducibility and performance of MCs, their various limits wanted to evaluate. Evaluation limit set for result of percent weight gain at GREC stage; percent yield; entrapment efficiency; particle size, size distribution, zeta potential; morphological evaluation; flow property; accelerated stability. Besides limit set for result of *in vitro* release and release kinetic; *in vivo* performance; *in vivo-in vitro* correlation studies, statistical treatment of data, and so on [18]. MCs also subject to assay of VPC, acid tolerance, bile tolerance, bile and acid tolerance and assimilation of cholesterol test [59]. The flow cytometry and immunofluorescence method may be follow to assess viability of immobilized or encapsulated VPC in a rapid way [60, 61].

FINAL PRODUCT EVALUATION

Developing PCMP, with success in marketplace, involves costly and multistage method. Besides, involve assessment of microbial and physical stability, safety, sensory acceptance, cost of health benefit, and other essential sensible properties [5, 26, 57].

Globalization of trade, and associated regulation, on the other hand, compels the manufacturers, for setting up uniform and scientifically acceptable quality and safety standards. Contamination of food borne pathogenic bacteria considered as a major challenge for safety and security. Known organisms based food hazards, arising out during processing, handling, or distribution as well heightens the safety issue. Handling issues and laws about contamination and food safety, major challenges, have to overcome while marketing food PCMP [62].

Issues about contamination and safety may be address through constitution and execution of hazard analysis critical control point regulations. Besides, evolutions of protocol have to done to identify high-risk food and processes, and for preventing problems and their recurrence [62]. Contamination of food borne pathogenic bacteria could detect with high-performance impedance bacteria biosensors [63]. According to current guidelines use of antimicrobials and thermal processing, efficient in inactivating the microbial and virus contamination, considered being ineffective in improving safety. Thus, alternative technologies ought prerequisite to overcome inactivation linked safety issue. Chemiresistive immunosensors applied for direct detection of viruses with high sensitivity and specificity [64]. Balancing

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inactivation and maintenance of sensory properties as well challenges the processes [62]. Optical chemical sensors and biosensors, having applicability, in mitigating food safety and security problems could employ [17].

Sensory analysis considered a prerequisite for customer espousal and a decisive step in evolution of PCMP. This acts as a measure for expecting product changes has an impact on marketplace, which links evolution of product with marketplace [1, 26]. Quantitative descriptive analysis (QDA), discriminative tests, affective tests, free choice profile, time-intensity analysis methods followed, with preference, for getting sensory performance of PCMP. Open-ended question, free-listening, check-all-that-apply approach, sorting, multivariate adaptive regression splines, survival analysis method, and internal preference mapping methods are alternative method of sensory testing [1, 26]. Discriminative tests (DCT) mark existence or absence of difference between samples. DCT shows whether two samples are different or analogous one, also quantify the degree of their similarity or dissimilarity with reference one. DCT may be a triangular test, paired-comparison test, duo–trio test (for comparison of two samples), or difference from control test (for more than two samples). Affective sensory tests point out extent of consumer likeliness or unlikeliness for them in hedonic scales, structured with 5, 7, or 9. Affective sensory tests report either their preference ranking (for more than two samples) or degree of likeliness (acceptance). The QDA and the free choice profile find out sensory profile with intensities of all descriptors in them. Time-intensity analysis finds out the intensity of a unique descriptor with time. Among all sensory test method, the QDA is put-up with preference [1, 26].

However, for convenience MCs loaded in a suitable PCMP such as capsules, tablets, suspensions, creams, powders, ice cream, juice, nutrient and chocolate bars, and so on. Basing on the intended effect and use, they are got evaluated according to existing laws and must confirm stability test. PCMP presented as drug, cosmetic must comply prevailing rules on Drugs, and Cosmetics, while that presented, as food must comply Food rules [11]. Interactions of PRs and prebiotics could assess by analyzing the proteomic profile with a μ-2DE system while rapid assessment of viability can do with flow cytometry [60].

PACKAGING

The packaging plays an important role in preserving PCMP's quality and safety, which can made amenable to point out quality, safety, and security, by devising active and or smart packages [17]. However, finished PCMP packed, by tradition, in glass containers, as PCs survival in glass bottles ought to be superior to in plastic bags. Biopolymer coated papers will promising packaging systems. Further, chemical or biosensors based smart-packages, ought to be more worthwhile, which can signal the food spoilage, packaging failure and contamination.

Biopolymer coating on paper packages, results active packages (biodegradable), will be worthwhile over conventional synthetic paper coatings. Formula of biopolymer-coating material enables incorporation of various additives that ought to improve its packaging efficiency. Extensive study ought to needed to optimize compositions of active coating materials and evaluate packaging efficiency of biopolymer coating materials. Extended and further research wanted for picking up information on their interactions with PCs and additives and effect on organoleptic features. Biopolymer paper-coating materials, under concern are caseinates, whey protein isolate, isolated soy protein, wheat gluten, zein, chitosan, carrageenan, alginate, starch, and so on [65].

Chemical or biosensors based packing will help in solving safety issue but increase cost of the product, distinctly, while their efficiency yet to found out [17, 63, 64, 66]. Packaging system, under consideration, ought to be use after carrying exposure assessments of leach food contact substances, complying specifications and limit [57].

FUTURE RESEARCH TRENDS IN PROBIOTICS AND PROBIOTICS DELIVERY

Scientific study afoot to evaluate blend of PRs strain inhibiting and or displacing pathogens for specific microbiota aberrancies coupled to disease risk. Their usefulness for specific target linked human health is also under study [67-70]. Research was continuing to develop low-cost PRs strain and their blend to treat or prevent specific disease through adhesion or targeting of pathogens [71, 72]. Systematic trials, upheld for proving therapeutic effect and mechanisms of action, of existing and or newer strains, species, or genus [72-74].

Enduring researches aimed to identify and characterize existing strains of PRs, identify strain-specific results, find out best doses needed for projected effects, assess VoPC during GI transit, and so on. Research directed to prolong shelf life; conserve VoPC during processing, storage, and GI transit phase; optimize adhesion capacity; develop active-packages; and evolving proper manufacturing, handling, and packaging techniques [7, 67-70].

PROSPECTIVE ACTION PLANS

These days, innovation considered as business mantra or slogan. Daily proclaim brought in, by business pundits, that ability to continue innovation will be only hope for resulting business survival. Therefore, evolution of PCMP carried over to fulfill the consumer's expectancy and wish which considered as challenging affairs. Their evolution bit-by-bit thought provoking the researchers, performing in both scientific and applied-field of research. Research in product design progressed to make best formula, through method of optimization. Scientific way of optimizing the method involves determination of optimal levels of key ingredients, essential to get product with suitable sensory and physicochemical characteristics, extended shelf life, chemical stability, and reasonable price. The method of optimization ought to be a difficult task, especially when several causes needed to be achievable, associated with many features.

Design of PCMP, a multistage method, involves several rudiments including huge funds. Several rudiments play crucial role in supervising their marketplace success and survivability. Amongst them, consumer acceptance a key issue, who must convinced and agreed to pay for the coupled useful claims, such as relish and fit. Therefore, PCMP must have suitable sensory and nutritional appeal, and worthwhile properties [1]. Global and territorial guidelines and legislation mandated for providing information to the consumers about a wholesome diet and for improving the availability and affordability of nutritious food according to their choice.

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Manufacturers, ought to be responsible for supplying products complying prevailing guidelines [5, 57]. Considering discussed fact following strategy adopted for developing them, following PEN.

Single or combination multiple PRs strains ought to select basing on projected worthwhile effects [14, 75]. These have to co-encapsulate with prebiotics basing on synbiotic effect. Single or combinations of multiple prebiotics, ought to selected, basing on synbiotic effect [76]. After selecting single or combination multiple strain, and prebiotics or their combination, additives and PEN having backing from regulators to select.

GREC material or CMFM, however, should selected depending on wished physicochemical and surface properties of MCs. Their selection method as well limited with incompatibility and thermo-liability issues. Thus, correct CMFM, GREC materials, additives and packaging materials, to select. It is a hard task to find a single GREC material or CMFM, which can enclose VPC and will result MCs which will excel the potential for marketability of PCMP.

Physicochemical properties of CMFM and GREC material, properties of core material, concentration of CMFM solution influence performance of MEC, for VPC. Besides, early concentration of PCs in formulation slurry, and limits and conditions of PEN influences the same. These rudiments have to selected, adopted, or fixed, in a judicious way [7]. CMFMs and GREC material having consent from regulators, compatibility with other ingredients, and release-controlling and synbiotic feature have to use preferably. These features of CMFMs and GREC material will synergize effect of MCs and PCMP. Coating method having regulatory consent, which will result effective GREC, should employed. Concentration of CMFM solution and solution of GREC material influences the size and size distribution of MCs, efficiency of encapsulation, and effectiveness of GREC, so need attention. Cell loading-efficiency of MCs improves with increase in early cell concentration in the dispersion.

Simple and continuous PEN having low processing cost and time, low iPVLP, superior scale-up potentiality and regulatory support should adopt. Causes influencing PEN efficiency, about MCs shape-size and iPVLP wants checking. Among discussed PEN, FCAP considered being convenient one followed by EVPM. This associated with their ease, duplicability, lessened iPVLP, lesser processing cost, and regulatory consent. Alternative to these, DCEM, following compression at pressures below 90 MPa, can adopt. The use of SDM with inclusion of suitable protectant in formula can be a wise choice. GREC of MCs, preformed, preferentially, has to done with FBP. Now, instrumentation for PEN unable to give large quantities of uniform sized MCs, with dwindled iPVLP, having aptness for industrial applications [7].

Control limits have to confirmed, in judicious and scientific way, to get product with ideal and wanted performance. Laid quality control and quality assurance limits followed strictly during PEN, GREC, and production. Evaluation of performance for finished product should carry over according to regulatory wants, and shelf life ought to assign with valid stability data in proposed packages. The storage conditions wanted to decided, in scientific way, a limit critical for preserving effectiveness of PCMP [7]. Besides, an efficient and smart package has to used, which can preserve quality of PCMP and can signal efficacy and safety of packaged material [17]. Labeling want on strain specificity and number of VPC at end of shelf life ought to complied, according to laws in force, with encouraging clinical, safety, and stability data [9, 15].

CONCLUSION

Modern food is ever changing, with space and time, and turning complex day-by-day. Professionals of this field must work together and with industry, regulatory authorities and policy makers to address issues on PRs use. Effort should undertake to improve availability of cost-effective healthy product choices for consumers. Low-cost PCMP can result through evolution of low-cost probiotic strain or strains, and synbiotics combinations coupled with prevention or treatment of diseases or ailment.

Importance of PEN is moving up with time with an aim to present large numbers of PRs containing food products such as capsules, tablets, suspensions, creams, powders, ice cream, juice, nutrient and chocolate bars, and so on. MEC of VPC wants well-developed technology, precise machinery, and better delivery systems. Confirm efficacy and efficiency of PCMP through human clinical trials. Ascribe are necessary to design and develop equipment that can give precise and uniform MCs of VPC in large quantities with lessened iPVLP and high loading efficiency while upholding sensory and nutritional quality.

Future will evidence utility of PEN in devising PCMP that will deliver projected benefit in a cost-effective and sustainable way. Step to set up to arouse consumers of all educational levels about correct uses of PCMP, to deliver projected worthwhile effects to society.

CONFLICTS OF INTERESTS

No conflict of interest published about this paper.

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